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VARIATION IN SEED CHARACTERISTICS AND GROWTH FOR THISTLES (CARDUEAE: ASTERACEAE) IN CALIFORNIA AND OREGON

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ABSTRACT

Genera in the tribe Cardueae (Asteraceae) include both non-invasive and invasive species widely dispersed in the western U.S. Seed characteristics are important for seed dispersal, seedling growth, and seedling survival. We determined seed characteristics and their variation from natural populations of 22 taxa of Cardueae. We also measured C and N content of seeds for 17 taxa, and conducted a greenhouse growth experiment with five species from this group. We tested the hypothesis that it is possible to distinguish invasive species from non-invasive species based on these characteristics. Seed weight differed significantly ($P < 0.0001$) among taxa and varied by a factor of 24, from a mean of 1.48 mg for *Centaurea solstitialis* L. to 35.63 mg for *Cynara cardunculus* L. subsp. *flavescens* Wiklund. There were no significant relationships between status as an invasive species and explanatory variables based on logistic regression results ($P > 0.05$). Seed N, C, and C:N ratio differed significantly ($P < 0.0001$) among taxa, but these characteristics were not associated with invasiveness. Relative growth rate (RGR) for greenhouse grown plants ranged from 0.010 to 0.030 g g^{-1} day^{-1}. Linear regression results indicate that there was no significant relationship between seed weight and RGR or other measures of plant growth and condition and seed weight. For many of the taxa analyzed in this study, the information on seed weight, nutrient content, and growth measures (RGR, net photosynthesis rate) have not been previously reported.

Key Words: *Carduus, centaurea, Cirsium, Cynara, Silybum.*

The tribe Cardueae Cass. (as described in Flora of North America) is one of the largest tribes in Asteraceae, consisting of over 2500 species worldwide (Barkley et al. 2006). In North America, Cardueae is represented by only two native genera: *Cirsium* Mill. (ca. 80 species) and *Saussurea* DC. (three species) (Keil 2006). California has a particularly high diversity of *Cirsium* species. The second edition of The Jepson Manual recognizes 39 native taxa (species and infraspecific varieties) (Baldwin et al. 2012). Despite this high amount of native diversity, California has accumulated a number of exotic Cardueae taxa. The second edition of the Jepson Manual (Baldwin et al. 2012) lists the following thistle genera in tribe Cardueae as fully naturalized in California: *Acroptilon* Cass., *Arctium* L., *Carduus* L., *Carthamus* L., *Centaurea* L., *Cynara* L., *Silybum* Vaill., *Volutaria* Cass., and several exotic species of *Cirsium*.

A plant's life history and the way it allocates limited resources to growth, defense, and reproduction can affect population dynamics and invasiveness. Differences in seed characteristics (e.g., size, weight, nutrient content) strongly affect demographic rates (i.e., propagule production, germination rates, seedling survivorship) and may help identify factors associated with invasiveness in native and exotic taxa. The size and nutrient content of seeds is important for seed dispersal, seedling growth, and seedling survival. When a seed germinates, growth is initially limited by the quantity of nutrients contained within it (Hendry and Grime 1993). Plants from smaller seeds appear to have higher relative growth rates than those from larger seeds (Marañón and Grubb 1993; Gross 1994). One theory has proposed that small seed size is a characteristic that may identify some invasive species (Rejmánek 1995; Grotkopp 2002); however, this does not always appear to be the case (Daws et al. 2007; Forcella 1985). In some cases, smaller seeds have greater dispersal abilities, but this depends on the dispersal mechanism (wind, water, vertebrate, ant, ballistic) and on plant height (Thomson et al. 2011). In the presence of granivores, small seed size may be advantageous as small seeds may not be as easily detected or handled as larger seeds (Henderson 1990). In contrast, seedlings from larger seeds may have a greater survival probability (Stanton 1984; Dalling and Hubbell 2002).

Despite the important role, seed size has in the survival and growth of plants there is little information on seed characteristics for several California and Oregon taxa of the Cardueae. Of the 22 taxa examined in this report we were only able to find information on seed size and its variation for four taxa. These include the invasive plant, *Centaurea solstitialis* L., the potential seed oil bioenergy crop, *Cynara cardunculus* L., the weedy *Silybum marianum* (L.) Gaertn., and *Cirsium vulgare* (Savi) Ten. Thus, this manuscript presents data on seed weight and its variation from natural populations of 22 taxa of Cardueae. We also present data on the nutrient content (C, N) of seeds for 17 taxa from this group and the results of a greenhouse experiment with five species from this group. We use this information to test the hypothesis that within tribe Cardueae it is possible to distinguish invasive species from non-invasive species based on seed traits (Ordonez et al. 2010). As noted by Ordonez et al. (2010) the approach of comparing traits of invasive and native species within a group of related species to identify traits associated with successful plant invasions has been informative.

Materials and Methods

Seeds were collected by hand from 22 Cardueae taxa at various sites in California and three sites in Oregon between 1998 and 2002, except that one collection was made in 1988 (Table 1). Nomenclature of these taxa follows Baldwin et al. (2012) unless otherwise noted. Flower heads were placed in paper bags and returned to the California Department of Food and Agriculture laboratory in Sacramento where seeds were removed. Seeds were transferred to small envelopes and stored under dry conditions at room temperature. Each seed was weighed individually to 0.1 mg (N = 73–943 seeds per taxon). For 17 taxa either five or 10 seeds from each taxon (216 seeds for *Centaurea solstitialis*) were analyzed for C and N content (percent of dry weight) using a Perkin-Elmer Model 2400 CHN analyzer with acetanilide used as the standard. We calculated the C:N ratio in order to assess the suitability of seeds as food items by comparing them with a value of 17. For example, C:N values greater than 17 may indicate less desirable food items for insects (McMahon et al. 1974; Karban and Baldwin 1997).

Elementary statistics (mean, coefficient of variation, standard error, median, skewness, and kurtosis) for seed weight for each taxon (across sample dates and sample sites) were calculated using the univariate procedure in SAS (2011). The skewness and kurtosis indices were included because they indicate the degree to which a frequency distribution departs from a normal distribution (Sokal and Rohlf 1995). Previous workers use the skewness statistic to summarize size distributions of plants within a population and as such, it could be applied to a population of seeds as well (Rejmánek et al. 1989; Petersen et al. 1990). Herrera (2009) has suggested that skewness and kurtosis may be important in describing plant phenotypes, especially for species producing large numbers of seeds. Statistical tests of the null hypothesis that the skewness index and the kurtosis index were not different from zero were performed with a two-tailed t-test as described by Sokal and Rohlf (1995). We calculated an unbalanced analysis of variance using the GLM procedure in SAS to test the hypothesis that seed weight, C, N, and C:N ratio did not differ across taxa. Effects were considered significant at the 0.05 level.

We tested the hypothesis that seed characteristics could identify invasive species using logistic regression. We calculated logistic regression with the binary species category, invasive/non-invasive status as listed by the California Invasive Plant Council (Cal-IPC 2006) as the dependent variable and mean seed weight, range of seed weights, skewness of seed weights, kurtosis of seed weights, or the coefficient of variation for seed weight as explanatory variables using the logistic procedure in SAS (2011).

Greenhouse experiment. This experiment was performed to determine the effect of seed size on growth rate of five species which varied in mean seed size (Table 2): *Centaurea solstitialis, Centaurea cyanus* L., *Cirsium douglasii* DC., *Cirsium andersonii* (A. Gray) Petr., and *Cynara cardunculus* L. subsp. *flavescens* Wiklund. The experiment was conducted from December 16, 2003 to May 26, 2004 in a greenhouse located at Davis, California. Typical conditions during the experiment were mean daily temperature 24°C and mean irradiance from a bank of fluorescent lamps 224 μmol m^{-2} s^{-1} with a 14:10 light dark cycle. The natural irradiance averaged 167 W m^{-2} s^{-1}, and increased from <20 W m^{-2} s^{-1} to >300 W m^{-2} s^{-1} during the experiment.

Twenty-five seeds of each taxon were placed in a small mesh bag and soaked in deionized-water for 24 hours. At planting, one seed of each species was placed in individual 1-L plastic pots (12 cm diameter by 12.5 cm tall) filled to a depth of 11 cm with a prepared clay substrate (one week prior to the day of planting, the clay was soaked in deionized water for five days). Pots were randomly assigned to positions on the greenhouse bench so that they were about 13 cm apart. There were a total of 125 pots (5 species × 25 pots). Hoagland's nutrient solution (Hoagland and Arnon 1950), with 75 mg N L^{-1}, was added once a week. Fifty ml Hoagland's solution was added in week one, 100 ml per week subsequently. Starting two weeks after planting, digital photos were taken for each pot and leaf areas determined from the pictures using an image analysis program (Sigma Scan Pro, Systat Software, Inc., San Jose, CA).

Every four weeks, five pots of each species (except *Cirsium andersonii*) were randomly select-

TABLE 1. TAXA, DATE COLLECTED, AND LOCATION FOR THE SEEDS USED IN THIS STUDY. All counties are in California except those followed by "(OR)" which are in Oregon. Species collections on the same date are from different sites within the county. The column labeled " Inv." presents the California Invasive Plant Council[1] rating for impact as an invasive plant: H = high, M = moderate, and L = limited. Dashed lines (—) indicate that the species is not currently considered to be invasive. [1] California Invasive Plant Council, Invasive Plant Inventory (CAL-IPC). Website http://www.cal-ipc.org/ip/inventory/index.php (accessed 4 June 2013) [2] HICKMAN, J. C. (ED.). 1993. The Jepson Manual: Higher plants of California. University of California Press, Berkeley, CA.

Taxon	Inv.	Date collected	County
Carduus pycnocephalus Spreng.	M	08/12/02	Solano
Centaurea cyanus L.	—	05/27/99	San Luis Obispo
		06/08/99	Shasta
Centaurea solstitialis L.	H	08/30/00	Siskiyou
Centaurea jacea L. nothosubsp. *pratensis* (W.D.J. Koch) Čelak.	M	08/16/00	Del Norte
Cirsium andersonii Jeps.	—	07/20/98	Nevada
		08/20/98	Nevada
		08/23/00	El Dorado
Cirsium brevistylum Cronquist	—	08/04/98	Humboldt
		08/16/00	Del Norte
		08/16/00	Humboldt
		07/19/00	Linn (OR)
Cirsium occidentale (Nutt.) Jeps. var. *californicum* (A. Gray) D. J. Keil & C.E. Turner	—	05/05/99	Kern
		05/05/99	Kern
		05/26/99	Santa Barbara
		07/13/00	Los Angeles
Cirsium occidentale var. *candidissimum* (Greene) J.F. Macbr.	—	07/22/99	Mono
		09/03/98	Plumas
		08/18/98	Shasta
		08/19/98	Modoc
		08/24/00	Mono
		08/29/00	Trinity
		08/23/00	Alpine
Cirsium cymosum (Greene) J.T. Howell var. *canovirens* (Rydb.) D.J. Keil	—	07/01/99	Nevada
		08/20/98	Nevada
		07/19/00	Lake
		08/23/00	Alpine
Cirsium crassicaule Jeps.	—	06/15/99	Kern
Cirsium cymosum (Greene) J. T. Howell var. *cymosum* (JFP-1)[2]		06/08/99	Siskiyou
		06/08/99	Siskiyou
		06/08/99	Siskiyou
		06/29/00	Lassen
Cirsium douglasii DC. (JFP-1)[2]	—	08/20/98	Nevada
		08/05/98	Humboldt
		08/20/00	Trinity
		08/17/00	Humboldt
		08/17/00	Humboldt
		08/17/00	Humboldt
		08/29/00	Trinity
		08/30/00	Trinity
Cirsium scariosum Nutt. var. *loncholepis* (Petr.) D.J. Keil	—	05/27/99	San Luis Obispo
Cirsium occidentale (Nutt.) Jeps. var. *occidentale*	—	06/15/99	San Luis Obispo
Cirsium ochrocentrum A. Gray	—	08/19/88	Modoc
		07/19/00	Lake
Cirsium remotifolium DC.	—	07/19/00	Linn (OR)
Cirsium scariosum Nutt. var. *scariosum* (JFP-1)[2]		07/01/99	Plumas
		02/09/98	Plumas
Cirsium undulatum Spreng.	—	07/12/98	(OR)
		07/18/00	Wasco (OR)
Cirsium occidentale (Nutt.) Jeps. var. *venustum* (Greene) Jeps.	—	06/15/99	Monterey
		06/15/99	San Benito
		06/15/99	Monterey
		06/15/99	Monterey
		06/15/99	Kern
		08/15/00	Mendocino
Cirsium vulgare (Savi) Ten.	M	08/12/02	Yolo
Cynara cardunculus L. subsp. *flavescens* Wiklund	L	07/10/00	Riverside
Silybum marianum (L.) Gaertn.	M	07/01/02	Yolo

TABLE 2. BASIC STATISTICS FOR 22 CYNAREAE (ASTERACEAE) TAXA. Statistics are number of samples (N), mean (Mean), coefficient of variation (CV), minimum (Min.), maximum (Max.), kurtosis (Kurt.), and skewness (Skew). The column labeled " Inv." presents the California Invasive Plant Council[1] rating for impact as an invasive plant: H = high, M = moderate, and L = limited. Dashed lines (—) indicate that the species is not currently considered to be invasive. Species are arranged from the smallest mean seed mass to the largest mean seed mass (mg). Differences in seed size among species were significant (P < 0.0001, unbalance analysis of variance calculated by PROC GLM). An asterisk (*) following kurtosis or skewness indicates that the value was significantly different from zero, P < 0.05. The complete scientific names are listed in Table 1. [1] California Invasive Plant Council, Invasive Plant Inventory (CAL-IPC). Website http://www.cal-ipc.org/ip/inventory/index.php (accessed 4 June 2013).

Taxon	N	Mean (mg)	CV %	Min.	Max.	Kurt.	Skew.	Inv.
Centaurea solstitialis	341	1.48	26.6	0.25	2.46	0.05	−0.27*	H
Cirsium brevistylum	190	2.07	24.8	0.74	3.51	0.11	−0.10	—
Centaurea jacea nothosubsp. *pratensis*	100	3.09	17.4	1.05	3.96	1.68*	−0.92*	M
Cirsium vulgare	200	3.13	15.4	1.25	4.14	1.45*	−0.82*	M
Cirsium scariosum var. *loncholepis*	100	3.98	21.5	1.97	5.65	−0.67	−0.43	—
Centaurea cyanus	130	4.13	21.7	1.60	6.40	0.32	0.19	—
Cirsium remotifolium	100	4.79	17.6	1.78	6.39	0.66	−0.47	—
Cirsium scariosum var. *scariosum*	136	4.88	30.1	1.43	8.36	−0.34	0.30	—
Cirsium crassicaule	100	5.41	19.3	2.62	6.96	−0.21	−0.77*	—
Cirsium cymosum var. *canovirens*	175	6.32	32.3	1.98	14.73	2.33*	1.17*	—
Carduus pycnocephalus	100	6.89	12.3	4.43	8.81	0.04	−0.42	M
Cirsium occidentale var. *occidentale*	73	7.10	20.4	3.57	9.46	−0.38	−0.41	—
Cirsium douglasii	310	7.22	33.8	1.62	14.78	0.20	0.01	—
Cirsium occidentale var. *californicum*	191	10.82	27.0	4.66	20.42	1.02*	0.88*	—
Cirsium occidentale var. *venustum*	251	12.99	22.8	4.89	20.89	−0.21	−0.14	—
Cirsium andersonii	156	13.03	32.0	4.23	22.85	−0.90*	0.28	—
Cirsium ochrocentrum	130	13.11	19.7	6.30	19.96	0.38	−0.08	—
Cirsium cymosum var. *cymosum*	220	14.11	28.8	5.62	24.09	−0.56	0.15	—
Cirsium occidentale var. *candidissimum*	411	15.58	26.1	1.59	25.08	−0.73*	−0.08	—
Cirsium undulatum	130	15.95	19.0	7.80	21.31	−0.16	−0.66*	—
Silybum marianum	100	23.09	14.5	10.89	29.63	1.12*	−0.58	L
Cynara cardunculus subsp. *flavescens*	130	35.63	27.7	11.06	48.29	−0.90*	−0.62	M

ed and harvested. Because *Cirsium andersonii* grew noticeably slower than the other species, two pots of *Cirsium andersonii* were harvested instead of five pots. The aboveground and belowground parts were separated and their fresh and dry weights determined. We calculated the relative growth rate (RGR) for each species using the logarithm of plant total dry weight as the dependent variable in linear regression against days since planting (Hunt 1982). For this, we used only data from the first four sampling dates since plant total dry weight declined after that.

Net photosynthesis measurements. We also measured relative leaf chlorophyll using a SPAD meter (Minolta 502, Spectrum Technologies, East Plainfield, IL). A SPAD meter measures the transmittance of red (650 nm) and infrared (940 nm) radiation through a leaf and calculates a relative meter reading, which reflects the chlorophyll content of the leaf (Uddling et al. 2007). For four species (*Centaurea cyanus*, *Cirsium douglasii*, *Centaurea solstitialis*, and *Cynara cardunculus* subsp. *flavescens*), on two dates (19 March 2004 and 5 May 2004) we measured net photosynthesis (estimated from carbon exchange rates, A_N) using a LI-COR 6400. All measurements were made between 10:00 and 14:00 hours. The first fully expanded leaf on a stem was measured to control for effects of leaf age. The measurement chamber was placed in the middle of the leaf on the leaf's upper surface. Conditions in the chamber were allowed to equilibrate until the stability parameter, total CV%, was near one, after which the appropriate measurements were recorded. Measurements were made at 1200 μmol m^{-2} s^{-1} (PAR), mean air temperature in the chamber when the measurements were made was 26.21°C on 19 March (standard deviation = 0.3, N = 14) and 28.0°C on 5 May (standard deviation = 0.7, N = 12). All of the SPAD and A_N measurements for a species were used to calculate the mean SPAD and A_N for each species. Mean values were used as dependent variables in linear regression versus mean seed weight for each species, to test the hypothesis that mean seed weight would predict values of these functional traits.

RESULTS

For the entire set of 22 taxa, seed weight varied by a factor of 24, from a mean of 1.48 mg for *Centaurea solstitialis* to 35.63 mg for *Cynara cardunculus* subsp. *flavescens* (Table 2). Results from analysis of variance indicate that the differences among taxa were significant (P <

0.0001). Based on the coefficient of variation *Cirsium douglasii* seed weight exhibited the greatest variation with *Cirsium occidentale* (Nutt.) Jeps. var. *compactum* Hoover second. The value for kurtosis index was significantly different from zero, for eight taxa, and the value for the skewness index was significantly different from zero, for seven taxa (Table 2). Mean seed weight for the six taxa considered as invasive (*Centaurea solstitialis, Centaurea jacea* L. nothosubsp. *pratensis* (W.D.J. Koch) Čelak., *Cirsium vulgare, Carduus pycnocephalus* L., *Silybum marianum,* and *Cynara cardunculus* subsp. *flavescens*) were among the smallest, mid-range, or the largest values for the 22 taxa examined in this study (Table 2). Accordingly, results of logistic regression of invasive status versus the explanatory variables (mean seed weight, range of seed weights, or the coefficient of variation for seed weight) revealed no significant relationships ($P > 0.05$) among the explanatory variables and status as an invasive plant species.

Differences among taxa for seed N, seed C, and seed C:N ratio were significant ($P < 0.0001$). The lowest value for seed N content was for *Cirsium undulatum* (Nutt.) Spreng. (1.77%) and the highest for *Cirsium andersonii* (3.32%) (Fig. 1). Seed N for two invasive species, *Carduus pycnocephalus* and *Centaurea solstitialis*, were greater than 2.5% and two others, *Cynara cardunculus* subsp. *flavescens* and *Silybum marianum*, had values near 2.5%. Tissue N for invasive species ranked intermediate among the range of taxa values. With respect to tissue C, two of the invasive species had the greatest values (Fig. 1). Tissue C for a third invasive species, *Silybum marianum* was sixth highest, and *Cynara cardunculus* subsp. *flavescens,* ranked fifth from the lowest. The C:N ratio ranged from 16.3–29.3. For *Centaurea solstitialis* and *Cynara cardunculus* subsp. *flavescens* C:N ratios were near 17, while C:N ratios for *Carduus pycnocephalus* and *Silybum marianum* were greater than that value. Based on the 95% confidence limits, the C:N ratio was greater than 17 for nine of the 17 taxa measured.

Growth experiment. Plant height and total leaf area increased at different rates following germination (Fig. 2). At the end of nine weeks, *Centaurea cyanus* plants were tallest and *Cirsium douglasii, Cirsium andersonii*, and *Centaurea solstitialis* were the shortest. Between 21 and 42 d after planting *Cynara cardunculus* subsp. *flavescens* had the greatest leaf area. After 49 d *Centaurea cyanus* and *Cirsium douglasii* had similar leaf areas to *Cynara cardunculus* subsp. *flavescens*. The smallest leaf area values were for *Cirsium andersonii. Centaurea solstitialis* leaf area was intermediate between these groups. The total dry weight of all species increased initially to maximum values around 10–14 wk after planting and then declined reflecting senescence (Fig. 3). *Cirsium douglasii* plants were the largest at the 14 week harvest. This species allocated more biomass to roots than the others (Fig. 3). Two species, *Centaurea cyanus* and *Centaurea solstitialis*, produced flowers during this experiment while the others did not. *Centaurea cyanus* produced flowers by six weeks after planting. Plant dry weight did not appear to be related to mean seed weight (Fig. 3). Relative growth rate (RGR) based on changes in dry weight (Fig. 4) was lowest for *Centaurea cyanus* (0.010 g g^{-1} day^{-1}) and greatest for *Cirsium douglasii*. (0.030 g g^{-1} day^{-1}). *Centaurea solstitialis* was second highest (0.024 g g^{-1} day^{-1}) however, there was no significant linear regression relationship between RGR and mean seed weight for the species examined (Table 3).

Net photosynthesis measurements. Net photosynthesis (A_N) varied from mean value of 8.4 μmol CO_2 m^{-2} s^{-1} for *Centaurea cyanus* to 12.5 μmol CO_2 m^{-2} s^{-1} for *Centaurea solstitialis* (Table 4). Under these conditions, differences among species were not significant (analysis of variance, $F_{3,25} = 1.48$, $P = 0.25$). There did not appear to be a significant relationship between initial seed size and net photosynthesis (Table 3). SPAD values ranged from 25.8 for *Centaurea cyanus* to 37.2 for *Cynara cardunculus* subsp. *flavescens* (Table 4). Differences among species means were significant (analysis of variance, $F_{3,21} = 3.94$, $P = 0.03$). There also did not appear to be a significant relationship between seed size and SPAD value (Table 3).

Discussion

In the present evaluation of 22 Cardueae taxa seed size characteristics were not significantly related to whether or not a taxon was considered invasive, suggesting that for these taxa seed weight is not a good predictor of invasiveness. Small mean seed weight was associated with invasiveness among species in the genus *Pinus* L. (Rejmánek 1995; Grotkopp 2002); however, this does not always appear to be the case, as others have associated increased seed size with invasiveness (Daws et al. 2007). For agricultural weeds, some of the most rapidly spreading weeds had relatively large heavy seeds (Forcella 1985).

The values for seed weight reported in this paper are similar to the few previous reports for these taxa. Mean seed weight for *Centaurea solstitialis* has been previously reported as 1.42 mg for seeds without a pappus and 1.18 mg for seeds with a pappus (Graebner et al. 2010). Widmer et al. (2007) reported that *Centaurea solstitialis* mean seed weights varied from 0.85–1.91 mg with a mean value of 1.22 mg for seeds

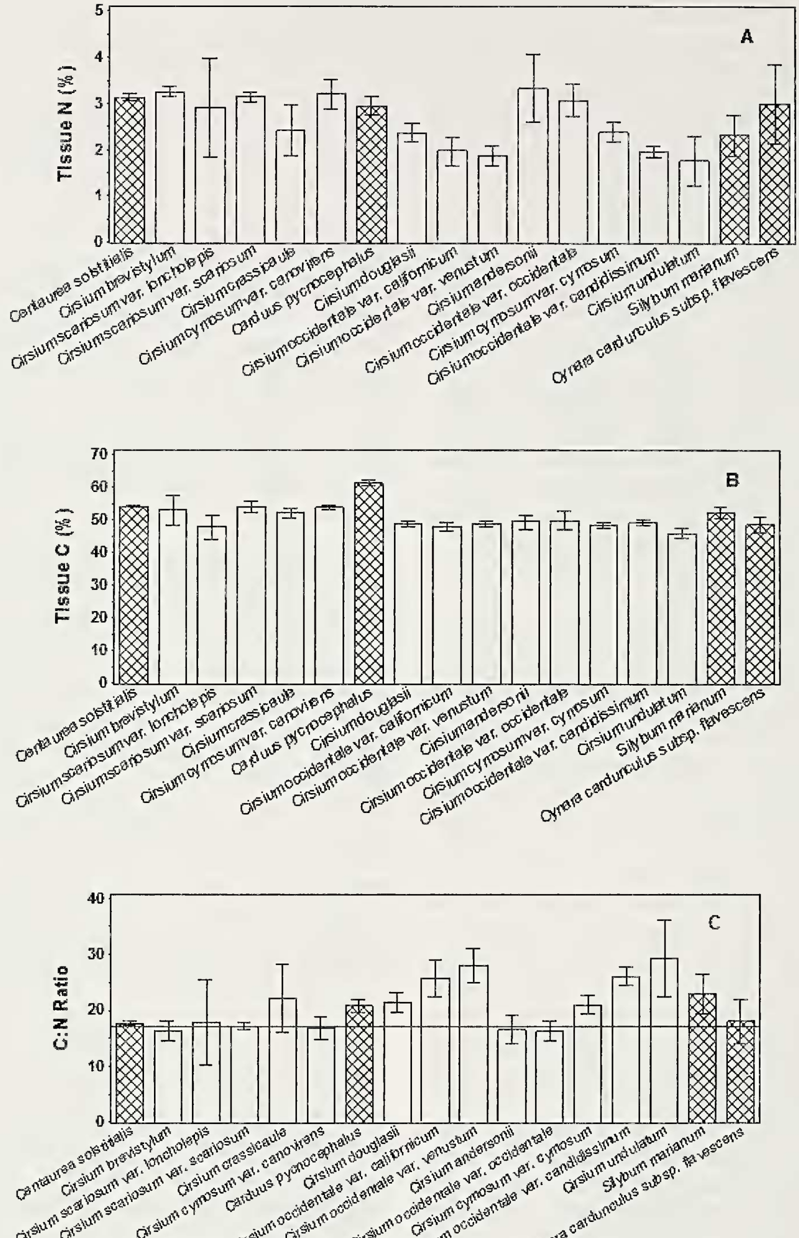

FIG. 1. Seed N% (A), C% (B), and C:N ratio (C) for 17 Cardueae taxa. Values are the mean ±95% confidence limits. Species are arranged from left to right in the order of increasing seed weight given in Table 2. Bars filled with cross hatch pattern are for species designated as invasive by California Invasive Plant Council (Cal-IPC 2006) (see Table 2).

collected at ten worldwide locations. Hierro et al. (2013) included a graph, which showed *Centaurea solstitialis* seed weights for seeds from Argentina and Turkey. Their values were quite close to the mean reported here (1.48 mg). Ghavami and Ramin (2008) reported that *Silybum marianum* seeds had an average weight of 23.9 mg for wild type plants or 21.4 mg for "Royston" type plants grown in a pot experiment in Iran. In the present study *Silybum marianum* seeds were 23.1 mg each on average. Foti et al. (1999) reported that individual *Cynara cardunculus* L. var. *sylvestris* Lam. seeds (wild type) were 19 or 21 mg per seed, and seeds of a related crop species (*Cynara cardunculus* L. var. *altilis* DC.) were 26 or 29.5 mg per seed in a two year field experiment in Italy. A report by Archontoulis et al. (2010) indicated a range of values for weight of individual *Cynara cardunculus* subsp. *flavescens* seeds of 26–56 mg. In our study the range was 11–48 mg per seed,

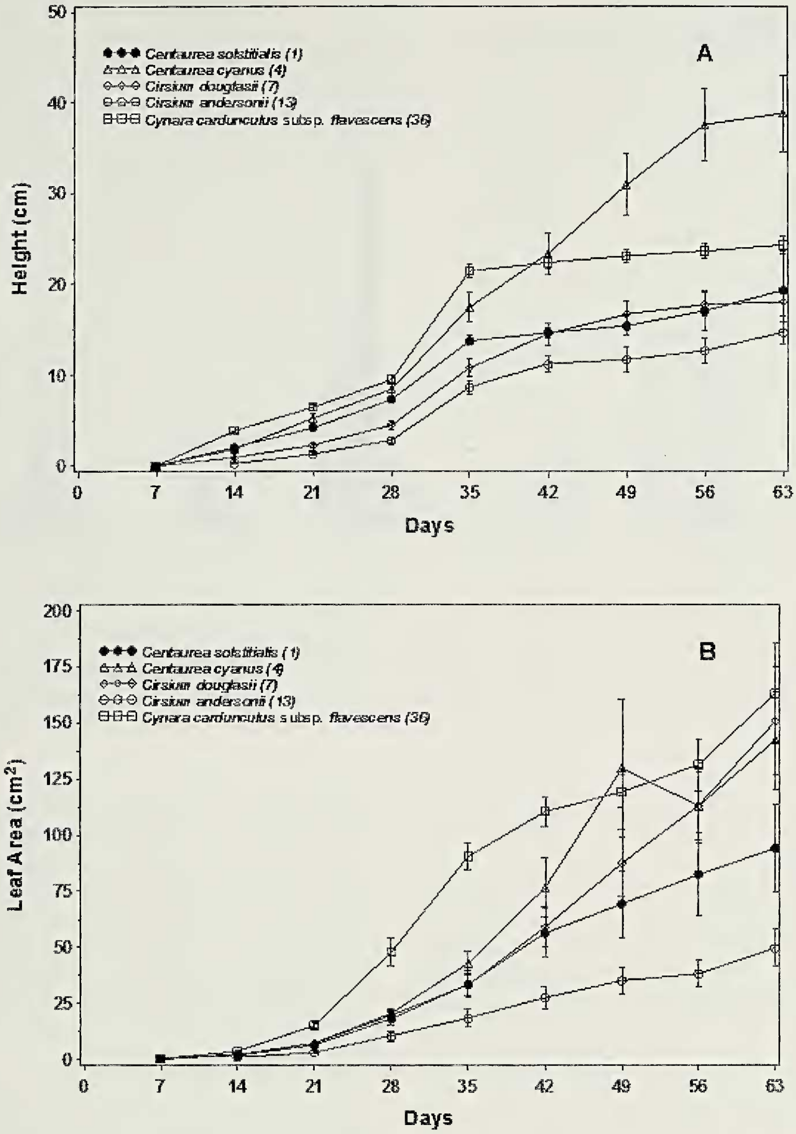

FIG. 2. Plant height (A) and leaf area (B) during the first nine weeks after planting for five taxa, which differed in seed size (*Cirsium andersonii, Cirsium cyanus, Cirsium douglasii, Centaurea solstitialis*, and *Cynara cardunculus* subsp. *flavescens*). Values are the mean ±1 SE, N for leaf area varied from six to 23 depending on the number of leaves present. N for plant height varied from two to five.

with an average value of 35.6 mg per seed. The slightly higher maximum values reported by Archontoulis et al. (2010) may in part be due to the fact that they are from experimental plots where the plants had been raised as crops, implying that some of the growing conditions had been optimized (i.e., weed control, fertilizers added). Considering that the values for *Cynara cardunculus* subsp. *flavescens* in our paper are from naturally growing populations growing under ambient conditions, the differences in seed size are small. To the best of our knowledge, the data on seed weight for the remaining taxa reported in this paper represent new information.

Variation in seed weight, as measured by the coefficient of variation (CV), in this study was similar to previous reports with the following caveat. Differences in how seed weight was determined make it difficult to compare variation in seed weight with previous studies. Many previous studies report seed weight per 100 seeds or some other number of seeds. Thus, the seed weight is actually a mean and as a result, any estimates of variation based on these means are under-estimates. The CVs for the present data were estimated from individually measured seeds. Nevertheless, the CVs for seeds from the 22 taxa reported here are in line with previous estimates.

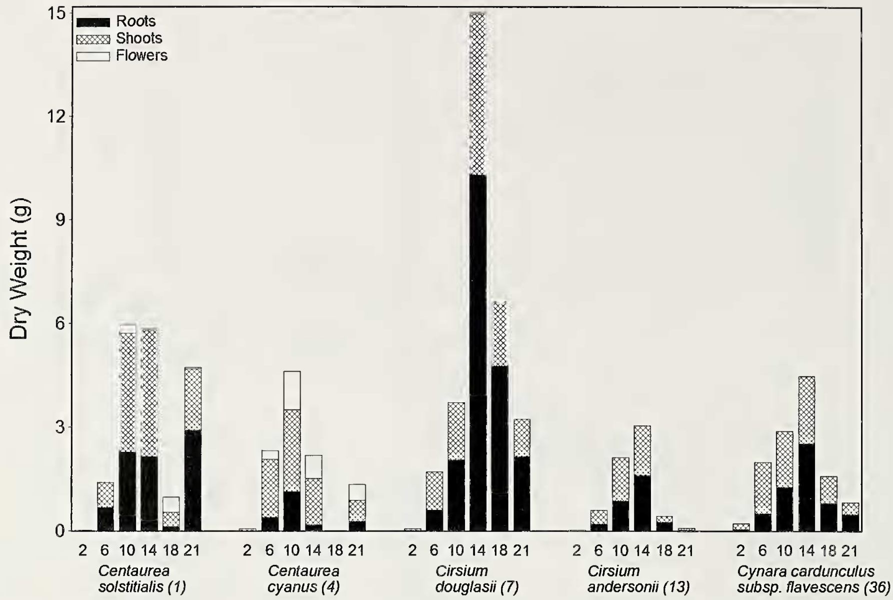

FIG. 3. Mean dry weight (grams) allocated to roots, shoots, and flowers over time (two, six, 10, 14, 18, or 21 wk after planting) for five taxa (*Cirsium andersonii*, *Cynara cardunculus* subsp. *flavescens, Cirsium cyanus, Cirsium douglasii*, and *Centaurea solstitialis*), which differed in seed size. The number in parentheses after the species name is the mean seed mass rounded to the nearest mg. Total dry weight (bar height) differed significantly due to species ($P < 0.0001$), time ($P < 0.0001$), and the interaction term, species by time ($P < 0.0001$) based on analysis of variance.

For example, CV for *Agropyron intermedium* (Host) Veauv. was 22% (Hunt and Miller 1965), for *Anthoxanthum odoratum* L. was 7.2% (Antonovics and Schmitt 1986), and for *Avena sativa* L. was 15.3% (Murphy and Frey 1962).

Significant values of skewness or kurtosis indicate that the seed weight distributions have more elongated distributions at one end or may have more than one peak. Either kurtosis, skewness, or both for seed weight distributions in this study were significantly different from zero, for 11 of the 22 taxa examined. In other studies, significant skewness or kurtosis indices indicate that environmental conditions influence seed weight more than maternal effects (Tungate et al. 2002; Tíscar and Lucas 2010). Seeds from

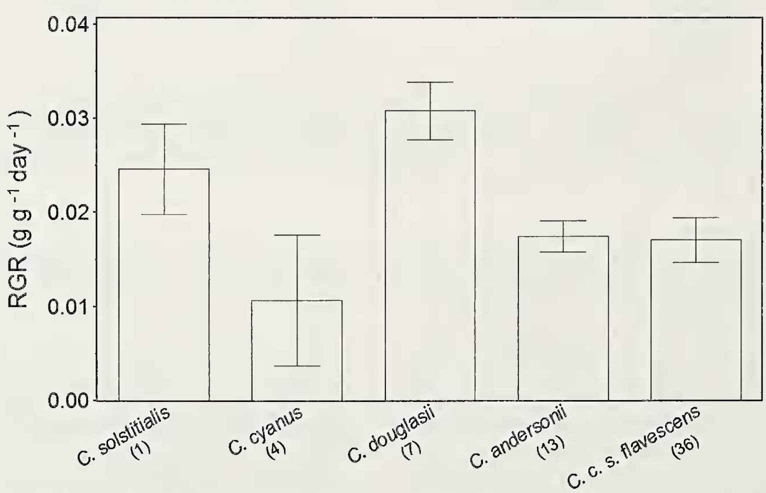

FIG. 4. Relative growth rate (RGR ±standard error) based on changes in dry weight over time for five species (*Cirsium andersonii, Cirsium cyanus, Cirsium douglasii, Centaurea solstitialis*, and *Cynara cardunculus* subsp. *flavescens*), which differed in seed size. The number in parenthesis to the right of the taxon name is the mean seed mass rounded to the nearest milligram (mg). RGR was not significantly related to seed mass based on linear regression (Table 3).

TABLE 3. LINEAR REGRESSION EQUATIONS RELATING PLANT GROWTH AND CONDITION CHARACTERISTICS TO SEED MASS. Units are as follows: RGR (g g^{-1} day^{-1}); leaf area (cm^2); SPAD (instrument units); mean plant height (height), (cm); A_N (μmol CO_2 m^{-2} s^{-1}); seed mass (mg). $P > F$ is the probability of obtaining a greater F-value and R^2 is the proportion of variation explained by seed mass.

Equation	DF	F-value	P > F	R^2
RGR = 0.022 − 0.00011 × seed mass	1, 3	0.09	0.80	0.04
Leaf Area = 41.3 + 0.77 × seed mass	1, 3	10.49	0.08	0.84
SPAD = 30.9 + 0.18 × seed mass	1, 3	0.70	0.49	0.26
Height = 10.8 + 0.05 × seed mass	1, 3	0.19	0.71	0.09
A_N = 10.61 − 0.048 × seed mass	1, 3	0.39	0.60	0.16

the taxa in this study came from locations, which encompass the entire north to south range examined in this study, and in several different years. No clear-cut pattern relating these factors to skewness or kurtosis of seed weight distributions is apparent from these data. An alternative explanation of significant skewness or kurtosis indices is that they indicate a strategy that is a form of bet hedging. That, is to say that seeds of different dispersal abilities (i.e., different weights) will likely land in a larger variety of microhabitats making it more likely that some will survive, especially in variable environments (Cohen 1966). While such a strategy may seem to favor successful invasions, there is no evidence for it from these data.

Tissue C and N values for seeds of Cardueae taxa reported here were similar to values reported for plant species in other published studies, 40% and 2–4%, respectively (Mengel and Kirkby 1982). Mattson (1980) reported that N content of different plant tissues ranged from 0.3–7.0% with highest concentrations (3–7%) occurring in growing tissues or in storage organs such as seeds. There is very little published information on the nutrient content of seeds of the taxa we analyzed. Foti et al. (1999) have published crude protein values for *Cynara cardunculus* subsp. *flavescens* seeds, which are equal to an N content of 3.21%. This is similar to the value for *Cynara cardunculus* subsp. *flavescens,* 3.0%, reported in the present study and within the 95% confidence limit shown in Figure 1. Seed C content for some of the seeds analyzed in this study was higher than the typical value for plant tissue (40%).

Information on seed nutrient content may be useful in understanding interactions between plants and the animals, which consume seeds. For example, plant tissue C:N ratios ≤ 17 are believed to be favorable to herbivores (Russell-Hunter 1970; McMahon et al. 1974). Values > 17 are believed to be N limited for invertebrate growth (Russell-Hunter 1970; McMahon et al. 1974). For example insect herbivores forced to compensate for feeding on low quality plant material (e.g., C:N ratio > 17) by consuming greater amounts of it may wear out their mouth parts more quickly (Karban and Baldwin 1997) or may ingest larger than desirable quantities of plant defense chemicals which may adversely impact their performance (Slansky and Wheeler 1992). Thus, it appears that some of the seeds examined may be a less desirable food item in some cases.

Under the conditions of the greenhouse experiment, only two species produced flowers. This implies that environmental controls of flowering (e.g., photoperiod) may differ among the five species examined. There is limited data for A_N (net photosynthesis) values for the four species measured in this study (Archontoulis et al. 2012; Dukes 2002). A_N values for these species at 1200 μmol CO_2 m^{-2} s^{-1} were toward the lower range of values reported for three energy crops whose light saturated A_N values ranged from 5 to > 35 μmol CO_2 m^{-2} s^{-1} (Archontoulis et al. 2012). The RGR values for these greenhouse grown plants ranged from 0.010–0.030 g g^{-1} day^{-1}. These values are toward the lower end of the range (0.01–0.13 g g^{-1} day^{-1}) previously reported for herbaceous species (Houghton et al. 2013). In the present study, there was no significant relationship between seed weight and RGR. Prior research has shown evidence that in some cases plants with larger seeds have lower RGR (Marañón and Grubb 1993) but not in all cases (Buckley et al. 2003). Other measures of plant growth and condition (e.g., mean plant height,

TABLE 4. NET PHOTOSYNTHESIS (A_N) AND SPAD VALUES FOR FOUR THISTLE SPECIES GROWN IN THE GREENHOUSE EXPERIMENT. Values are the mean, standard error, and number of leaves measured. Within each date species are sorted from top to bottom by seed size: 1, 4, 7, and 36 mg.

Species	SPAD (Units)	A_N (μmols CO_2 m^2 s^{-1})
Centaurea solstitialis	33.1 (1.9, N = 7)	12.51 (1.25, N = 8)
Centaurea cyanus	25.8 (5.32, N = 3)	8.40 (1.42, N = 4)
Cirsium douglasii	37.0 (1.6, N = 7)	10.61 (1.67, N = 8)
Cynara cardunculus subsp. *flavescens*	37.2 (1.6, N = 5)	9.29 (0.87, N = 6)

mean leaf area, mean leaf SPAD, mean net photosynthetic rate) were also unrelated to seed weight for the species examined (Table 3).

Relative biomass allocated to reproduction by plants including variation in seed size, can be influenced by environmental conditions (Thompson and Stewart 1981; Benech Arnold et al. 1992). Thus, information on seed size variation may contribute to understanding the distribution and abundance of Cardueae taxa. Some have suggested that functional characteristics such as seed size may be useful for predicting whether a species will be an invasive plant (Ordonez et al. 2010). The data on seed size variation, nutrient content, RGR, A_N, and leaf chlorophyll content for Cardueae taxa examined in this study do not support that hypothesis. This is perhaps not a surprising finding. Grime et al. (1988, see pp. 647) warned that while certain attributes may have predictive value in specific systems, this approach fails to be useful in all cases. They indicate that this is because 1) the same attribute may have different importance to different organisms and 2) natural selection affects more than one of a species' attributes at any one time resulting in a correlated set of attributes, which reflect ecological specialization. Results from recent studies agree with the warning and imply that it is unlikely that a universal suit of plant attributes exists, which will provide an explanation for the distribution of alien plant species (Tecco et al. 2010; Dawson et al. 2011).

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